E-D

Nonhuman Primate Models for AIDS

Patricia N. Fultz

From the Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama

Historically, animal model systems have been important components of biomedical research, and the same is proving true for research directed at the human immunodeficiency virus (HIV) pandemic. The most relevant and valuable models for studying infection by HIV-1 and HIV-2 and progression to AIDS involve infection of nonhuman primates with HIV-1, HIV-2, or some of the closely related simian immunodeficiency viruses (SIVs). The SIV macaque model has proven valuable in all aspects of AIDS-related research, with primary emphasis on defining pathogenic properties of lentiretroviruses and on testing novel approaches for prophylactic and therapeutic intervention. Because of certain limitations with the HIV-1 chimpanzee model, not the least of which is the expense involved, the use of HIV-naive chimpanzees should be limited to experiments related to vaccine development, an area for which their value has been demonstrated. Continued efforts to halt the spread of HIV infection and progression of HIV-related diseases will require further use of these animal models.

Animal models have played an important role in biomedical research and in the advancement of scientific knowledge in general. Of 82 Nobel Prizes for Physiology and Medicine awarded between 1901 and 1982, 71% were for research that involved the use of animals [1]. In the current war against AIDS, the use of animal models has contributed significantly to understanding the pathogenesis of and to developing vaccines against the causative agent, human immunodeficiency virus (HIV). Although several potential animal models exist, the most relevant for HIV are nonhuman primates infected with HIV-1, HIV-2, or various strains of the closely related simian immunodeficiency viruses (SIVs). To date, the models that have proven most valuable include chimpanzees (*Pan troglodytes*, HIV-1); cynomolgus monkeys (*Macaca fascicularis*, HIV-2); and rhesus (*Macaca mulatta*), pig-tailed (*Macaca nemestrina*), and cynomolgus macaque monkeys (SIV). This presentation is not meant to be a comprehensive review of all the various SIVs and animal models for HIV infection. Instead, the salient features of some specific systems used in my research program will be discussed to illustrate the primary value of each model.

HIV-1 Infection of Chimpanzees

The basic characteristics of HIV-1 infection of chimpanzees are similar to those of HIV-1 infection of humans, especially with respect to routes of transmission, development of humoral and cell-mediated immunity, and the fact that long-term persistent infections are established. Although no HIV-1–infected chimpanzee has developed symptoms of AIDS or AIDS-related conditions, hematologic abnormalities have been described. It is possible, therefore, that some of these animals eventually will develop overt disease.

Transmission of HIV-1 to chimpanzees. HIV-1 infections in chimpanzees can be established easily by intravenous inoculation of either cell-free or cell-associated HIV-1 and by transfusion of infected blood or blood products [2, 3]. Although data are more limited, perinatal infection can occur [4] and application of cell-free HIV-1 to the vaginal mucosa [5] or deposition of peripheral blood mononuclear cells (PBMC) from an unrelated HIV-infected chimpanzee to the cervical or vaginal mucosa in the absence of trauma can result in infection (unpublished data). Furthermore, HIV-1 does not appear to be easily transmitted between juvenile cagemates when only one animal of a pair is infected [6]; this situation simulates the lack of transmission by casual contact among humans.

Natural history of HIV-1 infection of chimpanzees. After exposure to an infectious inoculum, the natural history of HIV-1 infection in chimpanzees mimics that in humans. High levels of viral replication occur initially, as detected by quantification of infectious PBMC and isolation of cell-free virus from plasma. As HIV-1–specific serum antibodies are produced and antibody titers increase, viremia and numbers of infectious PBMC decrease [3, 5]. After ~6 weeks, cell-free virus is rarely isolated from plasma of infected animals, but virus can be isolated repeatedly from their PBMC (in >95% of attempts) throughout the resulting long-term infection [7]. This scenario is generally observed after inoculation of the two HIV-1 isolates most frequently used in chimpanzee studies: lymphadenopathy-associated virus type 1 (LAV-1 [LAI]) and human T-cell leukemia virus IIIB(LAI). However, the ease with which HIV-1 can be recovered from PBMC of infected chimpanzees is specific for a particular

Grant support: National Institutes of Health (AI-27136).

Reprints or correspondence: Dr. Patricia N. Fultz, Department of Microbiology, University of Alabama at Birmingham, 1918 University Boulevard, Birmingham, Alabama 35294.

Clinical Infectious Diseases 1993;17(Suppl 1):S230–5

1058–4838/93/1702–0033$02.00

virus isolate. For example, the SF2 (ARV-2) strain appears less infectious for chimpanzees: There are longer intervals between inoculation and initial time of virus recovery and sporadic, less frequent success in isolating virus from the infected animal's PBMC [8, 9].

At ~6 months after infection, overall serum antibody titers plateau and then remain relatively stable. However, loss of antibodies to *gag*-encoded proteins has been observed in some chimpanzees after extended periods of infection [7], just as occurs in HIV-infected persons. Neutralizing antibodies that are initially type specific but that broaden with time of infection to neutralize more diverse isolates can be detected [10, 11]. Other HIV-1–specific immune responses that have been described in infected chimpanzees include antibody-dependent cell-mediated cytotoxic activity, T cell proliferative responses to HIV-1 and recombinant HIV antigens, and major histocompatibility complex–restricted cytotoxic T cell activity [9, 10, 12–14]. The ability of chimpanzee $CD8^+$ lymphocytes to suppress HIV-1 replication in $CD4^+$ cells has been described [15]. Furthermore, since chimpanzees normally have more $CD8^+$ than $CD4^+$ cells, it is possible that $CD8^+$ lymphocytes may contribute to the failure of HIV-infected chimpanzees to develop AIDS-defining conditions.

Indications of disease in HIV-1–infected chimpanzees. While most of the >100 HIV-1–infected chimpanzees in the United States were exposed to the virus <4 years ago, some animals have now been infected for as long as 9 years. Although none has developed symptoms consistent with AIDS, there have been reports of persistent abnormalities in some animals. Two chimpanzees had generalized lymphadenopathy that persisted for ~7 months before spontaneously regressing [2, 16]. In addition, decreased numbers and percentages of $CD4^+$ lymphocytes, thrombocytopenia, hypergammaglobulinemia, and neutropenia have been described [3, 14]. In one animal with decreased numbers of peripheral blood $CD4^+$ cells (as low as 134/μL) and thrombocytopenia that persisted >18 months, plasma viremia and antigenemia were detected transiently, concomitant with onset of thrombocytopenia [14]. These results suggest a relationship between increased virus expression and disease symptoms; this correlation has been demonstrated for HIV-1 infection of humans and SIV infection of macaques (see below). Furthermore, analysis of the SIV model system also suggests a direct correlation between disease induction in a certain species and the cytopathicity of a virus isolate for $CD4^+$ lymphocytes of that species (see below). Several chimpanzees, including most of those with hematologic abnormalities mentioned above, are infected with an HIV-1 isolate that has been shown to be cytopathic for chimpanzee $CD4^+$ cells and to replicate efficiently in chimpanzee macrophages in vitro [17, 18].

Because of the close evolutionary relationship between chimpanzees and humans and the fact that there is a 10-year latent period between infection and diagnosis of AIDS for infected persons, it is probable that, if some chimpanzees eventually develop severe disease, the latent period will also be long. Furthermore, it is possible that the latent period for HIV-infected chimpanzees might be artificially extended because these animals are housed in isolation and are not routinely exposed to infectious agents as are humans. Because HIV-1 expression can be potentiated by cytokines and other microbial agents [19, 20], it is reasonable to assume that any infectious agent or injury that induces cytokines or inflammatory responses will also result in increased HIV replication, a factor known to correlate directly with disease progression [21–23].

Table 1. Chimpanzee human immunodeficiency virus type 1 (HIV-1) model in vaccine efficacy testing.

Advantages
Infection established with low doses of virus
Infection established with cell-free or cell-associated virus
Infection established by intravenous or mucosal routes
Humoral responses mimic those of humans
Virus easily recovered from peripheral blood mononuclear cells
Disadvantages
Cannot assess prevention of disease
Chimpanzees not equally susceptible to all strains of HIV-1

In fact, evidence to support this assumption exists. After immune stimulation of HIV-1–infected chimpanzees by administration of either recall or irrelevant antigens, rapid but transient increases in virus expression were detected by monitoring HIV-1–specific cellular and humoral immunity and by quantifying numbers of infectious PBMC [13, 24]. These studies suggest that prophylactic immunization against other pathogens or immunotherapeutic treatment of HIV-1–infected persons should be combined with antiretroviral therapy and that these persons should be monitored closely for changes in virus burden.

Value of the chimpanzee HIV-1 model. Even if chimpanzees eventually do develop AIDS, it would be unrealistic to use this species in pathogenicity studies because there are limited numbers of chimpanzees available; the expense involved in using sufficient numbers to generate statistically significant data is too great; and more importantly, the long latent period for disease development would require inordinately long follow-up times before useful conclusions could be made. Animals already infected with HIV-1, however, could be used to identify cofactors that facilitate manifestations of disease or to determine the effects of novel therapeutics on virus load [7].

The real value of chimpanzees is in the development of HIV-1 vaccines; these animals have played and will continue to play an important role in vaccine safety and efficacy trials. Although HIV-1 infection of chimpanzees is not an ideal model, it is the most suitable (table 1). Immunization of

Table 2. Nonhuman primate species from which simian immunodeficiency virus (SIV) has been isolated.

Species	Common name	Natural host	Disease*
Asian			
Macaca mulatta	Rhesus macaque	No	Yes
Macaca nemestrina	Pig-tailed macaque	No	Yes
Macaca fascicularis	Cynomolgus monkey	No	Yes
Macaca arctoides	Stump-tailed macaque	No	Yes
African			
Cercocebus atys	Sooty mangabey monkey	Yes	No
Cercopithecus aethiops	African green monkey	Yes	No
Cercopithecus mitis	Sykes' monkey	Yes	No
Papio sphinx	Mandrill (baboon)	Yes	U
Pan troglodytes†	Chimpanzee	U	U

NOTE. U, unknown.

* Experimental infection results in AIDS-like disease in species from which isolated.

† Chimpanzees are not simians and, therefore, isolates from this species should be designated CIV instead of SIV.

chimpanzees with various HIV-1 antigens, followed by challenge with cell-free or cell-associated virus, has demonstrated induction of protective immune responses [25–27]. Future studies will be required, however, to develop vaccines that elicit broadly reactive, group-specific immunity against the genetically diverse strains found worldwide; to determine whether mucosal immunity can be induced and protection against mucosal challenge can be achieved; and to optimize antigen presentation and immunization regimens to induce long-lasting protective immunity with a minimal number of vaccinations.

SIV Infection of Macaques

Diversity of SIV isolates. Lentiviruses closely related to, but distinct from, HIV-1 have been isolated from nine different nonhuman primate species, not all of which appear to be natural hosts (table 2). On the basis of comparisons of nucleotide sequences, HIVs and SIVs have been placed in five distinct subgroups, all of which are approximately equidistant from one another, with 40%–50% sequence homology. These subgroups include all of the SIV isolates from macaques and sooty mangabey monkeys together with HIV-2 isolates (SIV_{smm}, SIV_{mac}, and HIV-2); a more diverse group of isolates from African green monkeys (SIV_{agm}); isolates from mandrills (SIV_{mnd}); isolates from Sykes' monkeys (SIV_{syk}); and the HIV-1 and SIV_{cpz} isolates [28, 29]. It is highly likely that the number of distinct primate lentivirus subgroups will increase as more nucleotide sequence data are obtained from viruses isolated from other species.

Variation in pathogenesis of SIV infections. Because many SIV strains do not cause disease in their natural hosts (e.g., SIV_{smm}) but do elicit disease similar to human AIDS when inoculated into various macaque species, the opportunity exists to compare the natural history of infection in hosts for which the viruses are differentially pathogenic. In this way it may be possible to identify factors of the host or the virus that determine disease outcome. Additionally, infection of one Asian macaque species with different SIV isolates or of different macaque species with one SIV isolate can result in diverse pathogenicities with varying intervals of time from inoculation of virus to death due to AIDS-like disease. Thus, depending on the experimental question to be asked, one can optimize the information to be gained by selecting both the most appropriate macaque species and SIV isolate.

Two closely related SIV_{smm} isolates, SIV_{smm9} and $SIV_{smmPBj14}$, illustrate these points (table 3). Isolate smm9 was originally obtained from a naturally infected sooty mangabey monkey [30], whereas smmPBj14 was isolated from a pig-tailed macaque at the time it died of AIDS, 14 months after experimental inoculation of smm9 [31]. Although smm9 is the parental strain of smmPBj14, these two viruses exhibit very different pathogenicities. While smm9 elicits disease characteristic of lentiviruses, that is, a slowly progressive form [32], smmPBj14 induces a rapidly fatal disease. The acute disease caused by smmPBj14 is characterized, in general, by onset of bloody, mucoid diarrhea 4–8 days after inoculation, followed by death 2–4 days later. While the probable cause of death was originally thought to be due to metabolic acidosis and electrolyte imbalance resulting from dehydration, death frequently occurred despite fluid replacement therapy. More recent studies in which animals did not have diarrhea but still died 7–8 days after infection suggest that the acute disease may be similar to toxic shock syndrome and may result from inflammatory acute-phase reactant products induced by production of large amounts of virus and large numbers of proliferating lymphocytes (unpublished data). Gross pathology is characterized by severe gastroenterocolitis and generalized lymphadenopathy with

Table 3. Differential outcomes of persistent infections with two simian immunodeficiency virus (SIV) isolates.

Isolate/ species	Outcome	Time to death
SIV smm9		
Cercocebus atys	Asymptomatic infection	Natural
Macaca nemestrina	Chronic AIDS-like disease	7–14 months
Macaca mulatta	Chronic AIDS-like disease	14–>48 months
SIV smmPBj14		
C. atys	Acute fatal disease	10–13 days
M. nemestrina	Acute fatal disease	6–14 days
*M. mulatta**	Chronic AIDS-like disease	14–>40 months
	Acute fatal disease	7–14 days

* Rhesus macaques show extreme variation in susceptibility to smmPBj14, with only 30%–50% of infected animals developing the acute disease syndrome and dying.

diffuse lymphoid hyperplasia, which is most prominent in the Peyer's patches and other gut-associated lymphoid tissues [31]. Despite extreme differences in pathogenicities, the overall divergence of the smm9 and smmPBj14 nucleotide sequences is only 1.6% [33], indicating that minor mutations in lentivirus genomes can result in significantly increased virulence.

Because two closely related viruses (smm9 and smmPBj14) exhibit pathogenic extremes for mangabey monkeys and pig-tailed macaques, these two models provide a valuable system by which to identify correlates of disease. Comparison of the natural histories of smm9 infection in mangabeys and macaques revealed no major differences in the initial immune responses to the virus; however, macaques eventually progress to disease while mangabeys do not. The most striking differences were detected when virus burdens, that is, viremia and frequency of virus isolation from PBMC, were compared. Experimental infection of macaques elicited virus-specific immunity that, in some animals, can minimize virus burden for extended periods. In addition, in infected macaques progression to more severe disease correlated directly with increased levels of viremia. Surprisingly, asymptomatic infections in mangabeys were accompanied by very high virus loads, comparable to that seen in SIV-infected macaques with disease symptoms. Thus, the immune response of mangabeys appears unable to control SIV replication and dissemination but the animals do not develop disease [34].

The development of AIDS in both humans and monkeys is accompanied by loss of $CD4^+$ lymphocytes. Using in vitro assays, we determined whether SIV_{smm} was cytopathic for mangabey and macaque $CD4^+$ cells obtained from PBMC of normal animals. After infection of whole PBMC or $CD4^+$-enriched lymphocytes with smm9, preferential loss of macaque, but not mangabey, $CD4^+$ lymphocytes was observed during viral replication [34]. These results suggested that cytopathicity for $CD4^+$ lymphocytes, and not virus burden per se, may be important in eventual development of disease.

This hypothesis was tested further in similar experiments with the acutely lethal variant smmPBj14, which causes an identical disease syndrome in both mangabeys and macaques. Infection with this virus or its biologically and molecularly cloned derivatives (which also induce the acute disease) resulted in preferential loss of $CD4^+$ cells in PBMC from both species [35]. These results suggest that the degree of cytopathicity for $CD4^+$ lymphocytes may be an important determinant of disease. This concept is supported by the fact that SIV_{agm} does not appear to cause disease in its natural host and in vitro is not cytopathic for African green monkey $CD4^+$ cells [36]. If this apparent correlation is true, it would strengthen the idea that HIV-1–infected chimpanzees have the potential to develop disease.

Value of the macaque SIV model systems. Many facets of SIV infection of macaques are highly analogous to those of HIV-1 infection of humans. This is true for the natural history of infection and for specific manifestations of disease, including loss of $CD4^+$ cells, development of lymphomas [37], central nervous system involvement [38, 39], and types of opportunistic infections that occur [40–42]. Thus, the real value of this simian model is in defining viral and host factors that influence disease development, especially early after infection. An example of how the system can be manipulated was reported by Kestler et al. [43]. Using molecularly cloned viruses derived from an SIV_{mac} isolate, these investigators demonstrated a requirement for a functional *nef* gene for efficient replication and disease induction in macaques. Studies such as this may enable researchers to target specific HIV genetic regions or functions for intervention.

The macaque SIV model is also valuable for testing novel approaches and drugs for prophylactic and therapeutic efficacy [44, 45], including protection of fetuses [46], and for establishing important requirements for lentivirus vaccines [47, 48]. However, because potentially important differences may exist between immunologically relevant epitopes of SIV and HIV-1 [49], chimpanzees will continue to be used for definitive testing of HIV-1 vaccine candidates. The constraints associated with the use of chimpanzees may be alleviated by the recent construction of HIV-SIV chimeric viruses that express the HIV-1 envelope glycoprotein and infect macaques [50, 51].

Conclusions

Infection of chimpanzees with HIV-1 and macaques with SIV provide excellent animal models for studying all aspects of pathogenesis and prevention of HIV infection. Use of these models has resulted in substantial progress in understanding the interactions between lentiviruses and host cells that result in disease. Because no tissue culture system can adequately simulate the complexities of the body, these valuable animal resources will continue to be important in attempts to stop the spread of HIV infection and to prevent progression to AIDS.

Acknowledgments

The participation and contributions of colleagues in studies conducted by the author are gratefully acknowledged.

References

1. Leader RW, Stark D. The importance of animals in biomedical research. Perspect Biol Med 1987;30:470–85.
2. Alter HJ, Eichberg JW, Masur H, et al. Transmission of HTLV-III infection from human plasma to chimpanzees: an animal model for AIDS. Science 1984;226:549–52.
3. Fultz PN, McClure HM, Swenson RB, et al. Persistent infection of chimpanzees with human T-lymphotropic virus type III/lymphade-

nopathy-associated virus: a potential model for acquired immunodeficiency syndrome. J Virol 1986;58:116–24.
4. Eichberg JW, Lee DR, Allan JS, et al. In utero infection of an infant chimpanzee with HIV. N Engl J Med 1988;319:722–3.
5. Fultz PN, McClure HM, Daugharty H, et al. Vaginal transmission of human immunodeficiency virus (HIV) to a chimpanzee. J Infect Dis 1986;154:896–900.
6. Fultz PN, Greene C, Switzer W, Swenson B, Anderson D, McClure HM. Lack of transmission of human immunodeficiency virus from infected to uninfected chimpanzees. J Med Primatol 1987;16:341–7.
7. Fultz PN, McClure HM, Swenson RB, Anderson DC. HIV infection of chimpanzees as a model for testing chemotherapeutics. Intervirology 1989;30(suppl 1):51–8.
8. Fultz PN, Srinivasan A, Greene CR, Butler D, Swenson RB, McClure HM. Superinfection of a chimpanzee with a second strain of human immunodeficiency virus. J Virol 1987;61:4026–9.
9. Morrow WJW, Homsy J, Eichberg JW, et al. Long-term observation of baboons, rhesus monkeys, and chimpanzees inoculated with HIV and given periodic immunosuppressive treatment. AIDS Res Hum Retroviruses 1989;5:233–45.
10. Nara PL, Robey WG, Arthur LO, et al. Persistent infection of chimpanzees with human immunodeficiency virus: serological responses and properties of reisolated viruses. J Virol 1987;61:3173–80.
11. Goudsmit J, Debouck C, Meloen RH, et al. Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc Natl Acad Sci USA 1988;85:4478–82.
12. Eichberg JW, Zarling JM, Alter HJ, et al. T-cell responses to human immunodeficiency virus (HIV) and its recombinant antigens in HIV-infected chimpanzees. J Virol 1987;61:3804–8.
13. Fultz PN, Horaist C, McClure HM, Steimer KS, Dino D, Mawle AC. Postinfection immunization of human immunodeficiency virus–infected chimpanzees with recombinant HIV-1 *env* and *gag* antigens. In: Chanock R, Brown F, Lerner R, Ginsberg H, eds. Vaccines '89. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989:225–31.
14. Fultz PN, Siegel RL, Brodie A, et al. Prolonged $CD4^+$ lymphocytopenia and thrombocytopenia in a chimpanzee persistently infected with human immunodeficiency virus type 1. J Infect Dis 1991;163:441–7.
15. Castro BA, Walker CM, Eichberg JW, Levy JA. Suppression of human immunodeficiency virus replication by $CD8^+$ cells from infected and uninfected chimpanzees. Cell Immunol 1991;132:246–55.
16. Hu SL, Fultz PN, McClure HM, et al. Effect of immunization with a vaccinia–HIV *env* recombinant on HIV infection of chimpanzees. Nature 1987;328:721–3.
17. Watanabe M, Ringler DJ, Fultz PN, et al. A chimpanzee-passaged human immunodeficiency virus isolate is cytopathic for chimpanzee cells but does not induce disease. J Virol 1991;65:3344–8.
18. Gendelman HE, Ehrlich GD, Baca LM, et al. The inability of human immunodeficiency virus to infect chimpanzee monocytes can be overcome by serial viral passage in vivo. J Virol 1991;65:3853–63.
19. Nelson JA, Ghazal P, Wiley CA. Role of opportunistic viral infections in AIDS. AIDS 1990;4:1–10.
20. Matsuyama T, Kobayashi N, Yamamoto N. Cytokines and HIV infection: is AIDS a tumor necrosis factor disease? (editorial review). AIDS 1991;5:1405–17.
21. Schnittman SM, Greenhouse JJ, Psallidopoulos MC, et al. Increasing viral burden in $CD4^+$ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. Ann Intern Med 1990;113:438–43.
22. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 1989;321:1621–5.
23. Coombs RW, Collier AC, Allain J-P, et al. Plasma viremia in human immunodeficiency virus infection. N Engl J Med 1989;321:1626–31.
24. Fultz PN, Gluckman J-C, Muchmore E, Girard M. Transient increases in numbers of infectious cells in an HIV-infected chimpanzee following immune stimulation. AIDS Res Hum Retroviruses 1992;8:313–7.
25. Berman PW, Gregory TJ, Riddle L, et al. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature 1990;345:622–5.
26. Girard M, Kieny M-P, Pinter A, et al. Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus. Proc Natl Acad Sci USA 1991;88:542–6.
27. Fultz PN, Nara P, Barre-Sinoussi F, et al. Vaccine protection of chimpanzees against challenge with HIV-1–infected peripheral blood mononuclear cells. Science 1992;256:1687–90.
28. Johnson PR, Hirsch VM, Myers G. Genetic diversity and phylogeny of nonhuman primate lentiviruses. In: Koff WC, Wong-Staal F, Kennedy RC, eds. AIDS research reviews. Vol 1. New York: Marcel Dekker, 1991:47–62.
29. Hirsch VM, Dapolito GA, Goldstein S, et al. A distinct African lentivirus from Sykes' monkeys. J Virol 1993;67:1517–28.
30. Fultz PN, McClure HM, Anderson DC, Swenson RB, Anand R, Srinivason A. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). Proc Natl Acad Sci USA 1986;83:5286–90.
31. Fultz PN, McClure HM, Anderson DC, Switzer WM. Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM). AIDS Res Hum Retroviruses 1989;5:397–409.
32. McClure HM, Anderson DC, Fultz PN, Ansari AA, Lockwood E, Brodie A. Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. Vet Immunol Immunopathol 1989;21:13–24.
33. Courgnaud V, Laure F, Fultz PN, Montagnier L, Brechot C, Sonigo P. Genetic differences accounting for evolution and pathogenicity of simian immunodeficiency virus from sooty mangabey monkey after cross-species transmission to a pig-tailed macaque. J Virol 1992;66:414–9.
34. Fultz PN, Stricker RB, McClure HM, Anderson DC, Switzer WM, Horaist C. Humoral response to SIV/SMM infection in macaque and mangabey monkeys. J Acquired Immune Defic Syndr 1990;3:319–29.
35. Dewhurst S, Embretson JE, Anderson DC, Mullins JI, Fultz PN. Sequence analysis and acute pathogenicity of molecularly cloned $SIV_{SMM\text{-}PBj14}$. Nature 1990;345:636–40.
36. Honjo S, Narita T, Kobayashi R, et al. Experimental infection of African green monkeys and cynomolgus monkeys with a SIV_{AGM} strain isolated from a healthy African green monkey. J Med Primatol 1990;19:9–20.
37. Feichtinger H, Putkonen P, Parravicini C, et al. Malignant lymphomas in cynomolgus monkeys infected with simian immunodeficiency virus. Am J Pathol 1990;137:1311–5.
38. Lackner AA, Smith MO, Munn RJ, et al. Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. Am J Pathol 1991;139:609–21.
39. Chakrabarti L, Hurtel M, Maire M-A, et al. Early viral replication in the brain of SIV-infected rhesus monkeys. Am J Pathol 1991;139:1273–80.
40. Letvin NL, King NW. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Acquired Immune Defic Syndr 1990;3:1023–40.
41. Baskin GB, Murphey-Corb M, Watson EA, Martin LN. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 1988;25:456–67.
42. Baskerville A, Ramsay A, Cranage MP, et al. Histopathological changes

in simian immunodeficiency virus infection. J Pathol 1990;162:67–75.
43. Kestler HW, Ringler DJ, Mori K, et al. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. Cell 1991;65:651–62.
44. Watanabe M, Reimann KA, DeLong PA, Liu T, Fisher RA, Letvin NL. Effect of recombinant soluble CD4 in rhesus monkeys infected with simian immunodeficiency virus of macaques. Nature 1989;337:267–70.
45. Lundgren B, Bottiger D, Ljungdahl-Stahle E, et al. Antiviral effects of 3'-fluorothymidine and 3'-azidothymidine in cynomolgus monkeys infected with simian immunodeficiency virus. J Acquir Immune Defic Syndr 1991;4:489–98.
46. Fazely F, Sharma PL, Fratazzi C, et al. Simian immunodeficiency virus infection via amniotic fluid: a model to study fetal immunopathogenesis and prophylaxis. J Acquir Immune Defic Syndr 1993;6:107–14.
47. Wyand MS. The use of SIV-infected rhesus monkeys for the preclinical evaluation of AIDS drugs and vaccines. AIDS Res Hum Retroviruses 1992;8:349–56.
48. Gardner MB. Vaccination against SIV infection and disease. AIDS Res Hum Retroviruses 1990;6:835–46.
49. Javaherian K, Langlois AJ, Schmidt S, et al. The principal neutralization determinant of simian immunodeficiency virus differs from that of human immunodeficiency virus type 1. Proc Natl Acad Sci USA 1992;89:1418–22.
50. Li J, Lord CI, Haseltine W, Letvin NL, Sodroski J. Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. J Acquir Immune Defic Syndr 1992;5:639–45.
51. Sakuragi S, Shibata R, Mukai R, et al. Infection of macaque monkeys with a chimeric human and simian immunodeficiency virus. J Gen Virol 1992;73:2983–7.